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Comparative Analysis of the Schleicher and Schuell IsoCode Stix DNA Isolation Device and the Qiagen QIAamp DNA Mini Kit

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Efficient, rapid, and reproducible procedures for isolating high-quality DNA before PCR gene amplification are essential for the diagnostic and molecular identification of pathogenic bacteria. This study evaluated the Qiagen QIAamp DNA Mini Kit and the Schleicher and Schuell IsoCode Stix DNA isolation device for isolating nucleic acid. Buffer, serum, and whole-blood samples were spiked with *Bacillus anthracis* Sterne vegetative cells and *Yersinia pestis*, while water was spiked with *B. anthracis* Sterne spores. Although minimal variations in limit of detection occurred among matrices, both the IsoCode Stix extraction method and the Qiagen procedure have comparable detection limits.

Advances in molecular biology have led to the use of real-time PCR as an efficient and reproducible method for detecting bacterial and viral pathogens. PCR-based assays, designed to target specific nucleic acid sequences rather than relying on cultural and biochemical properties, offer high sensitivity and specificity (11, 14). These factors can be extremely important when rapid and accurate identification of pathogenic bacteria is required. Time-efficient and reliable methods for isolating high-quality nucleic acid are essential for the success of PCR-based technologies. The low concentration of DNA from pathogenic agents present in typical samples makes such applications necessary (3, 18, 22). In addition, a method with a flexible protocol applicable to numerous matrix types that is efficient at removing inhibitory substances found in clinical material that interfere with PCR amplification of the intended target is imperative (3, 12, 13, 15, 18, 20, 25, 26). Further, the proposed sample processing method should facilitate reproducibility, production of DNA for long-term storage, and minimal cross-contamination (10, 18, 19).

Various factors affect DNA recovery, including the degree of cellular lysis, binding of DNA to particulate material, and degradation or shearing of DNA (16). An optimal sample processing method should efficiently lyse resistant bacterial cell walls (gram positive) without indirectly damaging target DNA purified from more fragile (gram-negative) bacterial species (19). In addition, many current methods typically require multiple steps or specialized equipment, rendering them impractical for use with large sample numbers (1, 6, 14).

(These data were presented at the 2003 General Meeting of the American Society for Microbiology [S. R. Coyne, P. D. Craw, and M. P. Ulrich, Abstr. 103rd Gen. Meet. Am. Soc. Microbiol., abstr. C-195, 2003].)

This study was designed to compare the Qiagen QIAamp DNA Mini Kit and the Schleicher & Schuell IsoCode Stix DNA isolation device. Real-time PCR assays were used to measure the relative effectiveness of the Qiagen kit and the IsoCode Stix device in purifying and recovering bacterial DNA from clinical material including buffer, serum, and whole-blood samples. Gram-positive (*Bacillus anthracis* Sterne vegetative cells and spores) and gram-negative (*Yersinia pestis*) bacteria were tested to compare the two methods for DNA recovery and their compatibility with real-time PCR detection.

B. anthracis Sterne and *Y. pestis* CO92 were obtained from collections maintained at the United States Army Medical Research Institute of Infectious Diseases.

Tenfold serial dilutions of *B. anthracis* Sterne vegetative cells and *Y. pestis* CO92, beginning with approximately 10^6 CFU/ml, were prepared in phosphate-buffered saline (Sigma, St. Louis, Mo.), commercially available human serum (Pel-Freeze Clinical Systems, Brown Deer, Wis.), and human whole blood drawn into 5-ml EDTA collection tubes (Becton Dickinson, Franklin Lakes, N.J.). *B. anthracis* Sterne spores were prepared in molecular-biology-grade (MBG) water (Eppendorf, Westbury, N.Y.). Diluted bacterial samples were then enumerated by plating in duplicate on sheep blood agar medium (Remel, Inc., Lenexa, Kans.) to obtain actual concentrations for extraction.

For sonication, triplicate aliquots (100 μl) of *B. anthracis* Sterne spores diluted in MBG water were placed in an I-Core tube (Cepheid, Sunnyvale, Calif.) containing 30 to 40 mg of 106-μm and finer glass beads (Sigma). Samples were then placed in the lysis module of the Cepheid Microsonicator and sonicated for 15 s at the 70% power setting. Target DNA was purified using either the Qiagen QIAamp DNA Mini Kit (Valencia, Calif.) or the IsoCode Stix DNA Isolation Device (Schleicher & Schuell, Keene, N.H.).

DNA extraction using the Qiagen kit was carried out according to the manufacturer's instructions with minor modifications, as follows. Samples were prepared in triplicate by combining 100 μl of diluted bacteria with 80 μl of phosphate-buffered saline. This sample mixture was combined with 200 μl of buffer AL and 20 μl of proteinase K (17.8 mg/ml), followed by incubation at 55°C for 60 min. After incubation, 210 μl of ethanol (96 to 100%) was added, samples were mixed by vortexing, loaded onto a QIAamp spin column, and washed ac-

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14. ABSTRACT

Efficient, rapid, and reproducible procedures for isolating high-quality DNA before PCR gene amplification are essential for the diagnostic and molecular identification of pathogenic bacteria. This study evaluated the Qiagen QIAamp DNA Mini Kit and the Schleicher and Schuell IsoCode Stix DNA isolation device for isolating nucleic acid. Buffer, serum, and whole-blood samples were spiked with Bacillus anthracis Sterne vegetative cells and Yersinia pestis, while water was spiked with B. anthracis Sterne spores. Although minimal variations in limit of detection occurred among matrices, both the IsoCode Stix extraction method and the Qiagen procedure have comparable detection limits.

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TABLE 1. Extraction of *B. anthracis* Sterne vegetative cells[d]

Sample type	CFU/ml	Qiagen			IsoCode Stix		
		CFU/sample	Avg C_T[a]	Avg concn (fg)	CFU/sample	Avg C_T[a]	Avg concn (fg)
Buffer	5.0×10^5	50,000	26.33	11,736.93	20,000	27.51	5,772.78
	5.0×10^4	5,000	29.34	1,549.70	2,000	31.49	284.30
	5.0×10^3	**500**	**32.70**	**124.07**	200	33.84	53.65
	5.0×10^2	50	36.83[b]	6.54[b]	**20**	**37.63**	**4.26**
	5.0×10^1	5	0.00	0.00	2	0.00	0.00
	0	0	0.00	0.00	0	0.00	0.00
Serum	1.4×10^5	14,000	26.88	8,124.63	5,600	29.86	962.23
	1.4×10^4	1,400	30.11	768.02	560	32.61	127.98
	1.4×10^3	140	33.11	89.81	**56**	**35.82**	**13.48**
	1.4×10^2	**14**	**37.28**	**7.24**	5.6	36.63[c]	6.93[c]
	1.4×10^1	1.4	37.26[c]	4.42[c]	0.56	0.00	0.00
	0	0	0.00	0.00	0	0.00	0.00
Whole blood	4.5×10^5	45,000	28.92	1,892.86	18,000	34.01	112.67
	4.5×10^4	4,500	32.26	164.16	1,800	37.11	6.71
	4.5×10^3	450	35.24	19.27	**180**	**39.32**	**2.35**
	4.5×10^2	**45**	**37.96**	**3.12**	18	0.00	0.00
	4.5×10^1	4.5	0.00	0.00	1.8	0.00	0.00
	0	0	0.00	0.00	0	0.00	0.00

[a] C_T, cycle threshold.
[b] Two of three replicates produced a positive result.
[c] One of three replicates produced a positive result.
[d] Boldface type indicates the LOD.

cording to the manufacturer's instructions. Preheated (70°C) AE buffer (100 μl) was added to the column and incubated for 5 min at 70°C, and DNA was eluted by centrifugation at 6,000 × *g* for 1 min.

Nucleic acid purification using the IsoCode Stix procedure followed the manufacturer's instructions with few modifications. Each sample was prepared in triplicate, 10-μl aliquots were spotted onto the four triangular tips of the IsoCode Stix device (Schleicher & Schuell), dried for 15 min, and triangles were detached into sterile microcentrifuge tubes. MBG water (1 ml) was added to each tube, triangles were washed by pulse vortexing three times for a total of 5 s, and the wash water was removed. Sterile water was added (100 μl), ensuring complete submersion of the DNA-containing triangles, and nucleic acid was eluted by heating the mixture at 95°C for 15 min in an Eppendorf Thermomixer with an agitation setting of 6. After a brief centrifugation, eluates were removed and placed in sterile microcentrifuge tubes.

All nucleic acid was analyzed by real-time PCR with the Cepheid Smart Cycler. Reaction mixtures consisted of 20 μl of PCR mix and 5 μl of DNA template. DNA isolated from *B. anthracis* Sterne was analyzed with a pX01-specific assay (accession number M22589.1), and DNA purified from *Y. pestis* CO92 was analyzed with a pPCP1-specific assay (accession number X92856). The limit of detection (LOD) was determined when the PCRs were positive for each of the triplicate samples. In addition, an internal positive-control assay, developed at the United States Army Medical Research Institute of Infectious Diseases, was run on whole-blood and serum samples to monitor the presence of PCR inhibitors remaining in purified DNA samples (L. J. Hartman, S. R. Coyne, and D. A. Norwood, submitted for publication). Standard curves with purified nucleic acid data were constructed on the Smart Cycler to calculate the concentration of DNA obtained in experimental samples.

The Qiagen and IsoCode Stix comparison for *B. anthracis* Sterne vegetative cells in buffer, serum, and whole blood is seen in Table 1. Table 2 depicts the detection capability of the Qiagen and IsoCode Stix methods for *Y. pestis* CO92. The detection limits and recovery efficiency for *B. anthracis* Sterne spores in water determined with Qiagen and IsoCode Stix, with and without the incorporation of microsonication to enhance cellular lysis, are stated below. The LOD was greater than 20,000 CFU/sample (>2.0×10^5 CFU/ml) with the use of the Qiagen extraction without sonication, while the IsoCode Stix LOD was 8,000 CFU/sample (2.0×10^5 CFU/ml) with a recovery of 105.11 fg of DNA. When microsonication was used along with the sample processing methods tested in this study, the LOD was 2.0×10^3 CFU/ml for both Qiagen (200 CFU/sample) and IsoCode Stix (80 CFU/sample) and yielded DNA concentrations of 271 fg (Qiagen) and 39.09 fg (IsoCode Stix). PCR inhibitors were not detected in any of the purified DNA from serum and whole-blood samples tested in this study.

Although the sensitivity of a DNA extraction kit is important, many additional parameters for the clinical microbiology laboratory must be considered, including time required, typical sample types, cost per test, and the need for additional reagents (3, 14). In addition, a protocol that does not include specialized equipment or knowledge supports the routine isolation of DNA from a large series of samples (1). The diversity of clinical matrices (i.e., serum and whole blood) increases the complexity of samples to be processed for PCR detection (19). Thus, the method of template preparation is crucial to provide high-quality DNA lacking inhibitory factors (14, 20). In this study, both the IsoCode Stix and Qiagen methods effectively

TABLE 2. Extraction of *Y. pestis* CO92[d]

Sample type	CFU/ml	Qiagen			IsoCode Stix		
		CFU/sample	Avg C_T[a]	Avg concn (fg)	CFU/sample	Avg C_T[a]	Avg concn (fg)
Buffer	6.0×10^5	60,000	20.48	215,300.00	24,000	23.96	19,530.00
	6.0×10^4	6,000	23.73	23,030.00	2,400	27.06	2,300.00
	6.0×10^3	600	27.20	2,070.00	240	30.67	198.00
	6.0×10^2	60	30.73	197.00	**24**	**34.57**	**43.00**
	6.0×10^1	**6**	**33.89**	**21.00**	2.4	38.62[b]	1.00[b]
	0	0	0.00	0.00	0	0.00	0.00
Serum	1.1×10^6	110,000	19.59	397,890.00	44,000	24.87	10,430.00
	1.1×10^5	11,000	22.78	44,130.00	4,400	28.31	1,060.00
	1.1×10^4	1,100	26.30	3,920.00	440	31.41	119.00
	1.1×10^3	110	29.34	483.00	**44**	**34.96**	**11.00**
	1.1×10^2	**11**	**33.08**	**37.00**	4.4	39.14[c]	0.60[c]
	0	0	0.00	0.00	0	0.00	0.00
Whole blood	3.0×10^5	30,000	20.32	240,530.00	12,000	26.04	4,820.00
	3.0×10^4	3,000	23.60	25,220.00	1,200	29.82	355.00
	3.0×10^3	300	27.42	1,830.00	120	33.23	39.00
	3.0×10^2	30	30.64	198.00	**12**	**38.59**	**1.00**
	3.0×10^1	**3**	**33.70**	**24.00**	1.2	0.00	0.00
	0	0	0.00	0.00	0	0.00	0.00

[a] C_T, cycle threshold.
[b] Two of three replicates produced a positive result.
[c] One of three replicates produced a positive result.
[d] Boldface type indicates the LOD.

removed PCR inhibitors from serum and whole-blood samples.

The processing time varied between the IsoCode Stix procedure, which required approximately 45 min to process 12 samples, and the Qiagen kit, which needed approximately 90 min for 12 samples. In addition to the reduced time for purifying DNA, the IsoCode Stix procedure included only one reagent (MBG water), reducing the number of manipulations needed to obtain pure nucleic acid, improving the ease of sample handling, and minimizing the risk of cross-contamination. A unique feature of the IsoCode Stix is their impregnation with chelators and denaturants reported to retard bacterial and viral growth; inhibit nuclease activity, thus minimizing nucleic acid degradation; and release template DNA from organisms during processing (7, 8, 9, 13, 18, 21). Impregnated membrane-based technology has provided enhanced detection sensitivities compared to those with conventional preparations (18). This type of device also allows for storage of samples (applied to the card) and shipment at ambient temperatures, increasing its applicability to field studies (10).

In this study, PCR analysis on samples extracted using the IsoCode Stix DNA isolation device for template preparation provided LODs comparable to those of the Qiagen QIAamp DNA Mini Kit despite the reduced sample volume (40-μl maximum). However, based on quantification data generated by standard curves, the Qiagen procedure produced higher concentrations of DNA. In addition, sonicating the *B. anthracis* spores increased the LOD by 2 logs. The reason(s) for the decreased DNA recoveries with IsoCode Stix is unknown, but it may be a result of template DNA loss through degradation or trapping of nucleic acid in the paper matrix, which was previously shown (4). Bacterial cell DNA yields are reported with concentrations ranging from 0.5 to 5.36 fg of DNA per bacterial cell, depending on the species being analyzed (i.e., gram positive versus gram negative) (2, 5, 17, 23, 27). Based on an assumed average value of 9 fg of DNA per cell (24), the percent recovery for *B. anthracis* vegetative cells at the LOD with Qiagen was 2.8% for buffer, 5.8% for serum, and 0.8% for whole blood. In contrast, IsoCode Stix yielded lower percentages with 2.4% for buffer, 2.7% for serum, and 0.2% for whole blood at the LOD. Unlike *B. anthracis*, and as expected for gram-negative bacteria, increased percentages for both kits were obtained when analyzing extraction efficiencies for *Y. pestis*. The Qiagen kit yielded 38.9, 37.4, and 88.9% for buffer, serum, and whole blood, respectively, at the LOD. When IsoCode was used to purify nucleic acid from *Y. pestis*, the percent recovery at the LOD was 19.9% for buffer, 2.8% for serum, and 0.9% for whole blood. Sonicated *B. anthracis* spores revealed the same trend, with Qiagen providing a higher percent recovery (15.1%) than that of IsoCode Stix (5.4%) at the LOD. For unsonicated spores, IsoCode Stix yielded a value comparable to that for cells extracted in whole blood at 0.2%.

When combined with ease of handling, the IsoCode Stix device was sufficiently sensitive for PCR detection of the samples studied in this investigation. Preparing DNA from the IsoCode paper was rapid, simple, and reproducible, providing a more efficient method for template preparation.

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